



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<b>(21) International Application Number:</b> PCT/US91/04395 <b>(22) International Filing Date:</b> 21 June 1991 (21.06.91)  <b>(30) Priority data:</b> 541,527                      21 June 1990 (21.06.90)                      US  <b>(71) Applicant:</b> THE BOARD OF TRUSTEES OF THE LE- LAND STANFORD JUNIOR UNIVERSITY [US/ US]; Stanford University, Stanford, CA 94305 (US).  <b>(72) Inventors:</b> SHOPES, Robert ; 7575 Eads, #E, La Jolla, CA 92037 (US). OI, Vernon, T. ; 1259 Marilyn Court, Moun- tain View, CA 94040 (US). STRYER, Lubert ; 843 Sono- ma Terrace, Stanford, CA 94305 (US).		<b>(74) Agents:</b> ROWLAND, Bertram, I. et al.; Cooley Godward Castro Huddleson & Tatum, Five Palo Alto Square, 4th Floor, Palo Alto, CA 94306 (US).  <b>(81) Designated States:</b> AT (European patent), BE (European patent), CA, CH (European patent), DE (European pa- tent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), GR (Euro- pean patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> OLIGOMERIC IMMUNOGLOBULIN CONSTANT DOMAIN MUTANT WITH ENHANCED COMPLE- MENT-MEDIATED CYTOLYTIC ACTIVITY  <b>(57) Abstract</b>  Oligomeric immunoglobulins joined at the constant region at a specific site provide for enhanced cytolysis in conjunction with complement. The subject compositions are shown to be effective in complete cytolysis at substantially lower concentrations than required with the monomeric immunoglobulin. The immunoglobulins are mutagenized by providing a cysteine in the third heavy domain, whereupon expressing a dimeric product is obtained.		

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OLIGOMERIC IMMUNOGLOBULIN CONSTANT DOMAIN MUTANT  
WITH ENHANCED COMPLEMENT - MEDIATED CYTOLYTIC ACTIVITY

5

INTRODUCTION

Technical Field

10       The field of this invention is the use of  
immunoglobulins in binding to cells.

Background

15       A number of modalities, both therapeutic and in  
culture, utilizing monoclonal antibodies involve the  
purging of undesired cells. Immunoglobulin induced  
cytotoxicity can be attributed to a number of antibody  
dependent effector functions. One of these is the well-  
20       documented classical complement cascade initiated by an  
activated immune complex which leads to lysis of the  
antigen bearing cell. The molecular mechanism of the  
activation of complement has long been a source of  
speculation. Whether or not aggregation of antibody is  
25       sufficient is still an open question but it is generally  
thought that aggregation is necessary for initiation of  
the cascade. Aggregated antibody in the absence of  
antigen (e.g. heat treatment) can effectively activate  
the complement cascade. However, this is not useful in  
30       therapy. What would be useful is an immunoglobulin  
which displays little or no activation of complement in  
the absence of antigen binding and yields enhanced lysis  
of the target cell population. It would therefore be of  
interest to provide immunoglobulins or other proteins  
35       having specific binding capability as well as  
complement effector functions, with enhanced  
physiological activity.

Relevant Literature

- Waldmann (Ed.) Monoclonal Antibody Therapy;  
Progress in Allergy Vol. 45 (Karger, New York, 1988);  
5 Glassy and Dillman, Molecular Biotherapy 1,7 (1988),  
describe the use of monoclonal antibodies for the  
purging of undesired cells. There has been much  
speculation about the molecular mechanism of the  
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alteration of proteins has mainly focused on increasing  
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relationship of structure and function in various  
proteins. Creighton, Meth. Enz. 131,83 (1986); Falke  
and Koshland, Science 237, 1591 (1987); Matsumura and  
Matthews, Science 243, 792 (1989). Random  
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crosslinked non-immune rabbit immunoglobulin in the  
absence of antigen has been reported to display some  
constitutive activity. In complement fixation, the  
higher order aggregates are significantly more effective  
30 while monomers were inactive. Wright et al., Biochem.  
J. 187, 775 (1980). Earlier experiments imply that a  
higher order complex is required. Hyslop et al., J.  
Exp. Med. 131, 783 (1970); Baradun et al., Vox. Sang. 7,  
157 (1962). A synergistic effect in complement  
35 dependent lysis with two antibodies directed toward  
distinct epitopes on the same antigen has been reported.  
Elliott et al., Immunology, 34, 405 (1978); Howard and  
Hughes-Jones, Progress Allergy, 45, 1 (1988). The

effect of antigen density on antibody dependent complement mediated cytolysis has been examined theoretically (Gower and Segal, Mol. Immunol. 18, 823 (1981)) and experimentally (Alters et al., J. Immunol. (1989)).

#### SUMMARY OF THE INVENTION

Oligomeric immunoglobulins are provided for serving as effectors of complement mediated cytolysis. By replacing an amino acid with a cysteine in the constant region of the heavy chain, dimers can be obtained which provide for greatly enhanced complement effector function.

#### DESCRIPTION OF THE SPECIFIC EMBODIMENTS

Novel oligomeric immunoglobulin G compositions are provided, where an amino acid in the second or third, preferably third constant domain is replaced with a cysteine, whereby the oligomer may be produced in vitro from isolated monomer or in vivo by coupling during the process of synthesis or processing of the immunoglobulin. The immunoglobulins which are employed are those which have complement effector function, the human immunoglobulins being isotypes 1, 3 and 4. The compositions find use in providing for enhanced cytolysis of cells having epitopes to which the antibody binds. The cysteine may be used as a site for bonding to other linking molecules such as bifunctional compounds, e.g. bis-iodoacetyl imide bis-1,2-maleimido ethylene, or the like, which a time halo, olefin or other functionality may serve to bind the sulfur to act as a bridge.

The mutagenesis occurs at a codon in the second or third domain of the heavy chain, preferably the third, and at a codon which is higher than the 350th residue (see Kabat et al., Sequences of Protein of Immunological Interest, U.S. Dept. of Public Health NIH (1987)). Usually the mutagenesis will occur at a residue above

400, preferably at about 440 to 450, more preferably at about 444.

The mutation may be achieved by any convenient means, where the gene for the heavy chain may be  
5 isolated, particularly as cDNA. By employing in vitro mutagenesis, one can introduce the cysteine codon at the appropriate site. Alternatively, one may use a portion of the sequence encoding the antibody, conveniently the 3'-proximal region, where by use of appropriate  
10 primers, the sequence may be mutagenized using the polymerase chain reaction. Alternatively, the region containing the codon to be mutagenized may be restricted and a different sequence inserted having the appropriate codon at the appropriate site. The particular manner in  
15 which the cysteine is introduced is not critical to this invention and any convenient technique may be employed.

Once the heavy chain has been modified to introduce the cysteine, it may then be introduced into any  
20 appropriate host in conjunction with a light chain for expression of the desired immunoglobulin. The genes may be introduced into prokaryotic or eukaryotic hosts, particularly eukaryotic hosts, more particularly mammalian hosts, where the cells may be grown in  
25 culture or by employing transgenic mice may be grown in vivo. The light chain may be either a kappa or lambda chain, either the naturally occurring light chain naturally paired with the particular heavy chain, or a different light chain, when a different binding affinity  
30 may be of interest.

A wide variety of vectors are available for transformation of host cells for expression. The genes encoding the heavy and light chains may be on the same  
vector or may be co-transformed together. Vectors will  
35 normally involve an expression system which is functional in the host, where the expression system may be constitutive or inducible. A wide variety of expression regulatory systems, comprising enhancers,

promoters, and termination signals are available, being derived from viruses, e.g. SV40 early genes, naturally occurring genes, such as beta-actin, etc. Any convenient regulatory system, which provides the  
5 desired level of expression and ease of isolation may be employed.

The host cells may be any convenient host cell, particularly mammalian cells, such as monkey cells, mouse cells, etc. Usually, a marker will be provided  
10 for detection of cells which have been transformed with the genes encoding the heavy and light chains.

The particular manner of transformation is not critical to this invention, any convenient method being satisfactory. Once the cells have been transformed,  
15 they then may be selected in accordance with the nature of the marker and surviving cells cloned and expanded by conventional techniques. The cells may then be grown in culture and depending upon whether the signal sequence has been retained for the heavy chain, the  
20 immunoglobulins may be harvested from the supernatant or the cells harvested, lysed and the immunoglobulins separated from the cellular debris.

The mutagenized gene may be either the genomic gene or the cDNA gene. In the case of the genomic gene, the  
25 gene may be excised, the appropriate exon isolated and mutagenized, followed by reinserting the exon into its original site. For the cDNA, a fragment may be removed and mutagenized as by in vitro mutagenesis or the polymerase chain reaction, whereby the mutagenized gene  
30 may be obtained.

The gene will normally comprise a signal sequence for secretion of the immunoglobulin, the variable regions specific for binding to a particular epitope in  
35 conjunction with the light chain, the unmutagenized first constant region domain, the hinge region, the second constant region domain, normally wild type, and the third constant region domain, normally mutagenized and comprising the cysteine. While, for the most part,

the cysteine will be a substitution for another amino acid, it may also be an insertion at an appropriate site. Normally, the transmembrane integrator sequence will not be present, so that the immunoglobulins which  
5 form will be capable of secretion and will not be translocated to the membrane. The light chain will normally be the wild type light chain.

For the most part, the immunoglobulins will be human immunoglobulins, although the immunoglobulins may  
10 be from any mammalian host, particularly murine, feline, canine, equine, ovine, porcine, lagomorpha or primate. The dimeric antibody can be obtained directly from the expression host. However, it is found that the dimeric antibody is frequently obtained in conjunction with the  
15 monomeric antibody. By oxidizing the monomeric antibody in an appropriate aqueous medium, oligomers of the antibody may be obtained. The antibody compositions may be purified by conventional techniques, such as size-exclusion chromatography, gel electrophoresis, affinity  
20 chromatography, HPLC, etc.

The oligomeric antibody compositions may be used in a variety of ways. Of particular interest is the use of the subject antibody compositions in conjunction with a source of complement for cytolysis, where the antibodies  
25 bind to an epitope of a surface membrane molecule, particularly protein, saccharide, or lipid or combination thereof. The subject oligomers may be also used in therapy for binding to pathogens. In the case of microorganism cells, where the microorganisms are not  
30 invasive, the subject oligomers may be directed to epitopes on the surface of the organism. Thus, the subject oligomers may be used for the treatment of various pathogenic diseases, resulting from bacteria, fungi, protista, and the like. Where the organism is  
35 invasive, the subject antibodies may be directed to proteins or glycoproteins, which are expressed by the organism but are translocated to the surface membrane of the invaded cell. In this way, infected cells may be



killed, so as to avoid the proliferation and expansion of the pathogen. The subject compositions may also be used in the treatment of neoplastic cells, in both solid and non-solid tumors.

5           The subject compositions may be administered in conventional ways normally employed with antibodies. The subject compositions may be formulated in physiologically acceptable media, e.g. water, saline, phosphate, buffered saline, aqueous alkanol, or the  
10   like. The amount of the subject oligomers which is employed may vary widely, depending upon the particular purpose, the manner of administration, the frequency of administration, and the like. Generally, from about 1 pg to 10 mg per kilogram of host will be employed. The  
15   administration will normally be parenteral, particularly intravascularly, intramuscularly, or subcutaneously. The subject compositions may be administered as the only active ingredient or in combination with other physiologically active compounds.

20           The following examples are offered by way of illustration and not the way of limitation.

#### EXPERIMENTAL

##### Mutagenesis and an expression to yield the C444 mutant.

25           A genomic clone containing the constant domain exons of the heavy chain of human IgG<sub>1</sub> was pared down to a 2.3kb SalI - SphI fragment. This was inserted into pUC18 vector, in which the KpnI and SacI sites had been deleted in the polylinker. A SphI-ClaI-BamHI-(SphI)  
30   adaptor was inserted into the downstream SphI site. Insertion of a KpnI linker was used to delete a NaeI site between the C<sub>H</sub>2 and C<sub>H</sub>3 exons. A NaeI - NsiI fragment at the 3' end of the coding region of the C<sub>H</sub>3  
35   exon was replaced by a synthesized fragment which deleted the NsiI site, inserted a SmaI site and changed the codon for Ser<sup>444</sup> (in Eu notation) so as to code for Cys<sup>444</sup>. The resulting vector, pU18G1C444, was sequenced and the desired mutation verified. An undesired one

base deletion occurred. However it was 3' of the stop codon and would not affect protein synthesis. The mutagenized C<sub>3</sub> exon was cloned with the wild-type C<sub>1</sub>' hinge and C<sub>2</sub> hexons into a mammalian expression vector, pSV2DNSG1, which contained the heavy chain variable exon for anti-dansyl specificity. The resulting mutagenized heavy chain expression vector, pSV2DNSG1C444, was co-transfected into the murine myeloma SP2/0 cell-line with a mammalian expression vector coding for the anti-dansyl light chain with a human kappa exon. The resulting clonal cell-line, 143-17.12, produced a mutant dansyl-binding antibody with human constant domains of  $\gamma_1$  and kappa isotype (C444). Antibody was purified from three liters of supernatant, at approximately 3 mg/L, by affinity chromatography, dialysis and size-fractionation. Parental antibody, an anti-dansyl human  $\gamma_1$ , kappa with mouse variable domains (huG1), was produced in a similar manner. Cloning, sequencing and transfections were done as described by Maniatis et al., Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Laboratory Publications, 1982); Biggin et al., Proc. Natl. Acad. Sci. USA 80, 3963 (1983); Morrison and Oi in Handbook of Experimental Immunology (Weir ed), Vol. 2, 92.1 (Blackwell Scientific Publications, Palo Alto 1986).

#### Analysis of C444 Mutant by SDS-PAGE and gel-filtration chromatography.

A comparison was made of the C444 mutant and parental HuG1 by SDS-PAGE. The parent and mutant protein were run with no reductant on a 6% gel with high molecular weight standards. In a second run the two proteins were reduced with 20 mM dithiothreitol and run on a 10% gel with low molecular weight standards. The resulting gels were stained with Coomassie Blue, washed and copied with EDP paper (Kodak). The gel-filtration analysis of the C444 mutant employed a Superose 12 column (Pharmacia). Running buffer was 50 mM Tris,

150 mM NaCl (pH 7.8) at 0.25 ml/min. Native molecular weight standards and monomeric parental HuG1 ( $\gamma$ ) were run under the same conditions.

5     Antibody dependent complement mediated cytotoxicity  
      measurements.

      Titration of either parental HuG1 or isolated C444 dimer on sheep erythrocytes which were previously surface-labeled with dansyl<sub>28</sub>-BSA by a  $\text{CrCl}_3$  method was performed (Mishell and Shiigi, Selected Methods in Cellular Immunology (W.H. Freeman and Co., San Francisco 1980). Antibody ( $10 \mu\text{l}$ ) and sensitized cells ( $10 \mu\text{l}$  at  $4 \times 10^6$  cells/ml) were incubated at  $23^\circ\text{C}$  for 30 minutes in a U-bottom 96-well plate. Guinea pig complement ( $10 \mu\text{l}$  of an 1:1 dilution of Hemo-Lo (Cedarlane)) was added and incubated at  $37^\circ\text{C}$  for one hour. The cells were washed twice with buffer, lysed with  $200 \mu\text{l}$  water and split in four equal volumes into a flat-bottom 96-well plate. A chromogenic substrate for esterases,  $0.1 \text{ mg/ml}$  fluorescein diacetate (Molecular Probes), was added in a 1:1 volume. After development at  $37^\circ\text{C}$  for 1-2 h, the O.D. at 490 nm was read by a V-Max microplate reader (Molecular Devices). The O.D. reading was converted to number of cells and the percent lysis calculated. (Need more detail on the procedure). The buffer in all dilutions was 1x gel-HBS (Hardy, supra). Immunoglobulin was size-purified on a Superose column just prior to the experiment.

RESULTS

30       The C444 HuG1 antibodies were comprised of a 26kD (kilodalton) light and 53kD heavy chain as shown by SDS polyacrylamide gel electrophoresis (SDS-PAGE) of fully reduced samples. 50% of secreted C444 was typical immunoglobulin monomer, identical in size to the parental HuG1 as determined by non-reducing SDS-PAGE. The remainder of C444 had a higher molecular weight consistent with a covalent dimeric immunoglobulin. Non-denaturing, non-reducing gel-filtration chromatography

of C444 also indicated two distinct forms consistent with monomer and dimer forms of immunoglobulin. Upon reduction with 10 mM dithiothreitol, the C444 dimer was converted to monomeric form. Removal of the reductant by size exclusion chromatography allowed the formation of the C444 dimer. The data support that the dimeric form of C444 is attributable to a reducible bond. Pepsin digestion of the dimeric C444 indicated the formation of novel covalent but reducible, bonds between carboxy terminal domains. Cleavage of human IgG1 at residue 333 with pepsin yields a pFc' fragment which corresponds to essentially the carboxy terminal domain (Stanworth and Turner, in Handbook of Experimental Immunology (Weir ed.), Vol. 1, 12.1 (Blackwell Scientific Publications, Palo Alto 1986)). Digestion of C444 dimer with pepsin gave only a fragment corresponding to (pFc')<sub>2</sub>, while the parental IgG1 yielded a fragment corresponding to pFc'. However, the fragments became identical upon reduction. Thus the bond in the dimer was indeed between C<sub>H</sub>3 domains. The absence of any pFc' derived from the dimer indicated that the majority of C444 dimers are linked via two, rather than one, bond.

The data would support that the introduced cysteines at residue-444 in a pair of immunoglobulins forms an intermolecular disulphide linkage leading to a "dog-bone" shaped dimer.

The higher molecular weight form of the C444 mutant could be preparatively isolated by non-denaturing gel-filtration. This was shown to be equal to greater than 90% dimer by SDS-PAGE analysis, did not appear to contain oligomers greater than dimer and was stable for many weeks at 4°C. Isolated C444 dimer was used in an antibody dependent complement mediated cytotoxicity assay with parental HuG1. The dimer was clearly more effective than the monomer at the induction of lysis of target cells. Quantitatively, an initial concentration of 0.3 µg/ml of the C444 dimer gave 66% cytotoxicity. To

attain the same level of cytolysis with the parental HuG1, an initial concentration of 30  $\mu\text{g/ml}$  was required; a 200-fold increase in dosage on a molar basis.

5       The effectiveness of the dimer was shown not to be simply a mass action effect due to its tetravalent nature. At initial concentrations between 5 to 0.5  $\mu\text{g/ml}$ , where the complement activity difference is the greatest, the amount of C444 dimer bound to cell-  
10       surface antigen was less than 10% greater than that of HuG1 monomer and this negligible difference cannot account for the degree of lysis observed. Nor can the effect be due to the replacement of the Ser<sup>444</sup> residue in the mutant as monomeric C444 gave the same lysis  
15       results as monomeric HuG1. The data supports the conclusion that the greater activity of the dimer is attributable to the greater efficiency in the initiation of the complement cascade that leads to cell lysis.

For the purpose of comparison with immunoglobulin  
20       aggregation, chemically cross-linked monoclonal HuG1 anti-dansyl dimers derived from the parental monomers were prepared. Randomly linked anti-dansyl immunoglobulin dimer was prepared from monomer parental HuG1 by chemically cross-linking with the  
25       homobifunctional reagent SMPC (Pierce) (method B in Wright et al. (1980), supra) and isolating a 300 kD fraction by size-exclusion chromatography. In the presence of complement, the random cross-linked dimer at initial concentrations from 1 to 10  $\mu\text{g/ml}$  induced lysis  
30       of less than 10% ( $\pm 5\%$ ) of the labelled cells. This is significantly less than the 100% ( $\pm 5\%$ ) lysis given by the C444 dimer or even the 48% ( $\pm 8\%$ ) lysis observed for monomeric HuG1 at the same dosage.

The ability of the dimer to consume complement in  
35       the absence of antigen was measured with a cell-free complement fixation assay. (Hardy (1986), supra) In this assay monomeric immunoglobulin requires the binding of antigen for the formation of immune complexes to

initiate complement fixation. A small amount of pre-aggregated immunoglobulin, less than 5% of the total, can fix complement in the absence of antigen. In contrast to aggregated immunoglobulin, the amount of complement consumed constitutively by the C444 dimer was negligible, less than 5%; even at a 200-fold higher concentration than adequate for complete target cell lysis. The C444 dimer was effective at complement consumption in the presence of antigen.

It is evident from the above results, that greatly enhanced levels of in cytolysis of cells may be achieved by employing the oligomers of the subject invention. By providing for specific linking at a site in the constant region, particularly the C<sub>H</sub>3 domain, substantially smaller amounts of the subject compositions may be employed while achieving complete cytolysis. Thus, more efficient removal or destruction of cells can be achieved with the subject compositions as compared to monomeric immunoglobulin.

All publications and patent applications mentioned in this specification are indicative of the level of skill of those skilled in the art to which this invention pertains. All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

The invention now being fully described, it will be apparent to one of ordinary skill in the art that many changes and modifications can be made thereto without departing from the spirit or scope of the appended claims.

WHAT IS CLAIMED IS:

1. An oligomeric immunoglobulin comprising at least two immunoglobulin molecules other than IgM comprising a cysteine in the heavy chain constant region as a result of a mutation at a codon greater than the 350th codon, wherein said immunoglobulin molecules are joined by mean of said cysteine to form an intermolecular link.

2. An oligomeric immunoglobulin according to Claim 1, wherein said immunoglobulin molecules are IgG.

3. An oligomeric immunoglobulin according to Claim 2, wherein said IgG is of an isotype having complement effector function.

4. An oligomeric immunoglobulin according to Claim 2, wherein said mutation is substitution of a an amino acid at a residue in the range 440 to 450.

5. An oligomeric immunoglobulin according to Claim 4, wherein said residue is 444.

6. A DNA sequence encoding an immunoglobulin heavy chain subunit of other than IgM comprising a cysteine as a result of a mutation at a codon greater than the 350th codon.

7. A DNA sequence according to Claim 6, wherein said immunoglobulin molecules are IgG.

8. A DNA sequence according to Claim 7, wherein said IgG is of an isotype having complement effector function.

9. A DNA sequence according to Claim 7, wherein said mutation is substitution of a codon at a residue in the range 440 to 450.

10. A DNA sequence according to Claim 9, wherein said residue is 444.

11. A DNA sequence according to Claim 6 comprising at the 5' terminus a signal sequence.

12. A DNA sequence according to Claim 11, wherein said sequence is an immunoglobulin heavy chain signal sequence.

13. An expression cassette comprising in the order of transcription, a promoter, a DNA sequence encoding an immunoglobulin heavy chain subunit of other than IgM comprising a cysteine as a result of a mutation at a codon greater than the 350th codon, and a terminator sequence.

14. An expression cassette, wherein said DNA sequence encodes a signal sequence at its 5' terminus.

15. A eukaryotic host comprising an expression cassette according to Claim 14.

16. A eukaryotic host comprising an expression cassette according to Claim 13.

17. A method of preparing an oligomeric immunoglobulin comprising at least two immunoglobulin molecules other than IgM comprising a cysteine in the heavy chain constant region as a result of a mutation at a codon greater than the 350th codon, wherein said immunoglobulin molecules are joined by said cysteine to form an intermolecular cystine, said method comprising:  
growing a eukaryotic host according to Claim 16 in an appropriate nutrient medium, whereby said oligomeric immunoglobulin is expressed; and  
harvesting said oligomeric immunoglobulin.

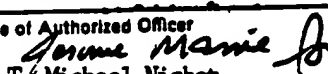
18. A method for producing complement mediated cytolysis, said method comprising:

bringing together (1) cells to be cytolysed, (2) an oligomeric immunoglobulin comprising at least two immunoglobulin molecules other than IgM comprising a cysteine in the heavy chain constant region as a result of a mutation at a codon greater than the 350th codon, wherein said immunoglobulin molecules are joined by said cysteine to form an intermolecular cystine, said oligomeric immunoglobulin being of an isotype to bind complement and specifically binding to a surface membrane molecule of said cells, and (3) complement; whereby said oligomeric immunoglobulin and complement bind to said cells and lyse said cells.



# INTERNATIONAL SEARCH REPORT

International Application No. PCT/US91/04395

<b>I. CLASSIFICATION OF SUBJECT MATTER</b> (if several classification symbols apply, indicate all) <sup>6</sup>		
According to International Patent Classification (IPC) or to both National Classification and IPC IPC(5): A61K 39/395, C07K 13/00; C12N 15/13; U.S.C1: 530/387; 536/27; 435/69.6		
<b>II. FIELDS SEARCHED</b>		
Minimum Documentation Searched <sup>7</sup>		
Classification System	Classification Symbols	
U.S.C1:	435/69.6, 965, 971, 972, 975; 530/387-390, 408; 536/27; 424/85.8, 85.91, 529-534	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched <sup>8</sup>		
Automated Patent System and Dialog Keyword Searches: complement?, mutant?, chimeric?, igl, immunoglob?		
<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT <sup>9</sup></b>		
Category <sup>*</sup>	Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>	Relevant to Claim No. <sup>13</sup>
Y	FASEB, vol. 4, issued 07 June 1990, Baird et al., "IgE has a bent conformation before and after binding to its high affinity receptor (F <sub>c</sub> E RI) on RBL cells", see abstract 2153.	1-18
Y	DE, A, 3,112,334 (Becker et al) 07 October 1982, see entire document.	1-18
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p><sup>*</sup> Special categories of cited documents: <sup>10</sup></p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"A" document member of the same patent family</p> </div> </div>		
<b>IV. CERTIFICATION</b>		
Date of the Actual Completion of the International Search		Date of Mailing of this International Search Report
23 September 1991		08 OCT 1991
International Searching Authority		Signature of Authorized Officer
ISA/US		 T. Michael Nisbet